Molecular Cloning

A LABORATORY MANUAL

T. Maniatis Harvard University
E. F. Fritsch Michigan State University
J. Sambrook Cold Spring Harbor Laboratory

Cold Spring Harbor Laboratory
1982

Molecular Cloning

A LABORATORY MANUAL

Printed in the United States of America
Book and cover design by Emily Harste

Front cover: The electron micrograph of bacteriophage λ particles stained with uranyl acetate was digitized and assigned false color by computer. *Thomas R. Broker, Louise T. Chow, and James I. Garrels*

Back cover: E. coli DH1 with fimbriae was negatively stained with phosphotungstic acid and the electron micrograph was digitized and assigned false color by computer. *Jeffrey A. Engler, Thomas R. Broker, and James I. Garrels*

Cataloging in Publications data

Maniatis, T.
Molecular cloning.

(A laboratory manual)
Bibliography: p.
Includes index.
1. Molecular cloning. 2. Eukaryotic cells. I. Fritsch, Edward F. II. Sambrook, Joseph. III. Title. IV. Series.
QH442.2.F74 574.87'3224 81-68891
ISBN 0-87969-136-0 AACR2

Researchers using the procedures of this manual do so at their own risk. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

HYBRIDIZATION OF SOUTHERN FILTERS

1. Float the baked filter on the surface of 6× SSC until it wets from beneath. Immerse the filter in the 6× SSC for 2 minutes.

2. Slip the wet filter into a heat-sealable plastic bag (e.g., Sears' Seal-n-Save).

3. Add 0.2 ml of prehybridization fluid warmed to 68°C for each square centimeter of nitrocellulose filter.

   Prehybridization fluid

   6× SSC
   0.5% SDS
   5× Denhardt's solution (see page 448)
   100 μg/ml denatured, salmon sperm DNA (see page 327)

4. Squeeze as much air as possible from the bag. Seal the open end of the bag with the heat sealer. Incubate the bag for 2–4 hours submerged in a water bath at 68°C.

   Often, small bubbles of air form on the surface of the filter as the temperature of the prehybridization solution rises to 68°C. It is important that these bubbles be removed by occasionally agitating the fluid in the bag; otherwise the components of the prehybridization fluid will not be able to coat the filter evenly.

5. Remove the bag from the water bath. Open the bag by cutting off one corner with scissors. Squeeze out as much prehybridization solution as possible.

6. Using a pasteur pipette, add the hybridization solution to the bag. Use just enough solution to keep the filter wet (50 μl/cm^2 of filter).

   Hybridization solution

   6× SSC
   0.01 M EDTA
   ^{32}P-labeled denatured probe DNA
   5× Denhardt's solution
   0.5% SDS
   100 μg/ml denatured, salmon sperm DNA

   Typical hybridization conditions for Southern filters are given in Table 11.1.

7. Squeeze as much air as possible from the bag. Seal the cut edge with the heat sealer so that as few air bubbles as possible are trapped in the bag.

TABLE 11.1 HYBRIDIZATION CONDITIONS FOR SOUTHERN FILTERS

DNA on filter	Sp. act. of probe DNA (cpm/μg)	Amount of probe added	Time of hybridization (hr)
Fragments of cloned DNA (~ 100 ng/fragment)	10^7	10^5-10^6 cpm (0.01-0.1 μg)	3-4
Total eukaryotic DNA (10 μg)	10^8	1×10^7 cpm -5×10^7 (0.1-0.5 μg)	12-16

8. Incubate the bag submerged in a water bath at 68°C for the required hybridization period.

9. Remove the bag from the water bath and quickly cut along the length of three sides. Using gloves, remove the filter and immediately submerge it in a tray containing a solution of 2× SSC and 0.5% SDS at room temperature.

   Note. Do not allow the filter to dry out at any stage during the washing procedure.

10. After 5 minutes, transfer the filter to a fresh tray containing a solution of 2× SSC and 0.1% SDS and incubate for 15 minutes at room temperature with occasional gentle agitation.

11. Transfer the filter to a flat-bottomed plastic box containing a solution of 0.1× SSC and 0.5% SDS. Incubate at 68°C for 2 hours with gentle agitation. Change the buffer and continue incubating for a further 30 minutes.

    Note. If the homology between the probe and the DNA bound to the filter is inexact, the washing should be carried out under less stringent conditions. In general, washing should be carried out at $T_m = -12$°C.
    The following relationships are useful:

    a. $T_m = 69.3 + 0.41 \cdot (G + C)\%$ (Marmur and Doty 1962)

    b. The T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatched base pairs (Bonner et al. 1973).

    c. $(T_m)\mu_2 - (T_m)\mu_1 = 18.5 \log_{10} \frac{\mu_2}{\mu_1}$

    where μ_1 and μ_2 are the ionic strengths of two solutions (Dove and Davidson 1962).

12. Dry the filter at room temperature on a sheet of Whatman 3MM paper.

13. Wrap the filter in Saran Wrap and apply to X-ray film to obtain an autoradiographic image (see page 470).

Notes

Hybridization may also be carried out in:

a. flat-bottomed plastic boxes.

b. buffers containing formamide. Each increase of 1% in the formamide concentration lowers the T_m of a DNA duplex by 0.7°C (McConaughy et al. 1969; Casey and Davidson 1977).